How Host Specific is Infection with Koi Herpesvirus (KHV) for Real?

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Key words: KHV infection, detection methods, nested PCR, non *Cyprinus carpio* species

Contrary to the theory of host specificity of herpesviral infections, channel catfish herpesvirus (CCV) is able to infect more than one species, e.g., blue catfish (*Ictaluris furcatus*), channel catfish (*Ictaluris punctatus)*, and white catfish (*Ictaluris catus*). Although African catfish (*Clarias gariepinus*), Asian catfish (*Clarias batrachus*), and other species are resistant to clinical CCV infection, CCV infected a mammalian cell line obtained from Hawaiian monk seal (*Monachus schauinslandi).* Due to these findings, we sought more host species in the framework of koi herpesvirus (KHV) infection (KHVI). KHV disease (KHVD) occurs only in *Cyprinus carpio* (common carp and koi). Among other species, we tested goldfish (*Carassius auratus*), crucian carp (*C. carassius*), tech (*Tinca tinca*), grass carp (*Ctenopharyngodon idella*), bighead (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), sheatfish (*Silurus glanis*), sturgeon species (*Acipenser gueldenstaedtii, A. oxyrhynchus, A. ruthenus*), and ornamental fish by different PCRs. All non *Cyprinus carpio* fish had never expressed any clinical sign of KHVD. Using different methods for DNA extraction (clumbs, fluid reagents) from different organs (organs, swabs, smears, blood, etc.), KHV DNA was detected in most of the experimentally or naturally-infected fish by nested PCR. Positive PCR results were confirmed by *in situ* hybridization using different probes, by sequencing of PCR products, or by immunofluorescence assay using polyclonal and monoclonal antibodies developed against KHV. In the same framework we tried to establish and test the sensitivity of non-lethal sampling methods, e.g., separation of leukocytes or gill swabs. Our aim was to exclude or detect KHVI in fish (carriers) that were in contact with KHV-infected carp or other fish affected by KHVI.

Major Losses of Wild Fish in the USA from a Novel Strain of Viral Hemorrhagic Septicemia Virus (VHSV)

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Key words: VHS, rhabdovirus, USA, type IVb

Viral hemorrhagic septicemia virus (VHSV) is among the most important viral pathogens of finfish. A member of the genus *Novirhabdovirus*, the VHSV virion is a bullet-shaped particle con-

taining a single-stranded, negative sense RNA genome of approximately 11,000 nucleotides. Genetic analysis reveals that isolates of VHSV fall into four genotypes that generally correlate with geographic location. North American isolates of VHSV are assigned to Genotype IV; however, in 2005, a new isolate identified as Genotype IVb was detected in fish from the Great Lakes. The type IVb isolate is the only strain outside of Europe associated with high mortality in freshwater species. The first epizootics attributed to VHSV IVb occurred in 2005 in fish from Lake St. Clair, Michigan, USA, and the Bay of Quinte on the northern shore of Lake Ontario, Ontario, Canada. Since that time, the virus has been associated with major mortality events involving many families of freshwater fish in 25 different species. As of 2007, the virus has been found in four of the five Great Lakes as well as the connecting waterways and several inland lakes in the states of New York, Michigan, and Wisconsin. Federal and State governments have enacted stringent new regulations designed to reduce the risk that the virus will spread to commercial aquaculture or to wild or cultured fish outside the Great lakes region. While it may be too early to make judgments on a seasonality of the disease, many of the mortality events appear to occur in the spring and early summer. We hypothesize that this may be due to environmental stressors, such as the changing (increasing) water temperature that occurs in the spring in combination with stress associated with spawning activity.

Iridovirus Infection in Cultured Tilapia (*Oreochromis* sp)

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Key words: tilapia, iridovirus, isolation

At Intervet Singapore (INS), we isolated iridoviruses from many cultured marine fish species such the humpback grouper, tiger grouper, potato cod, mullet, and Asian sea bass. Infected fish may or may not show visible external clinical signs (such as anemia) other than lethargy and the darkened appearance typical of sick fish. An enlarged spleen and swollen kidney can indicate iridovirus infection after elimination of the presence of any bacterial infection. An outbreak of *Streptococcus iniae* in cultured tilapia suggested concurrent viral infection. The spleen was typically very enlarged in infected fish and some fish also had exophthalmia and pale internal organs. Electron microscopy of spleen tissue revealed the presence of icosahedral viral particles typical of iridoviruses and this was confirmed by PCR analysis using primers against the iridovirus major capsid protein. Interesting, as with all other iridoviruses isolated at INS, the virus was not detected by PCR using the OIE primers for the red sea bream iridovirus (RSIV). Virus isolates from tilapia appeared to be genetically different in this respect and therefore we designated them non-RSIV like. The virus has so far been found only in relatively small tilapia up to 60 g. Tilapia iridovirus strains have been successfully isolated on an Asian sea bass brain (SBB) cell line developed at INS. These cultured viruses have been shown to kill tilapia under experimental conditions when injected intraperitoneally. This is the first report of any isolation of iridovirus from tilapia since an iridovirus-like agent in tilapia was first reported by D.G. MacGrogan et al. in 1998.

Re-Emerging *Oncorhynchus masou* Virus Disease (OMVD) of Rainbow Trout and Its Control Strategy

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Key words: *Oncorhynchus masou* virus disease, re-emerging disease, rainbow trout, disease control

Oncorhynchus masou virus (OMV) is a herpesvirus isolated from salmonid fish in Japan. OMV is much more pathogenic to kokanee, masu, and chum salmon than to coho salmon or rainbow trout. OMV disease (OMVD) involves oncogenic and skin ulcer conditions. During surveillance for virus isolation in northern Japan in 1978-2006, OMV was distributed widely and the infected species was masu salmon. From 1988, OMV was isolated from coho salmon and OMVD was a major problem in coho salmon pen culture in Tohoku district. From 1991, OMVD was found in rainbow trout in Hokkaido. Economic losses were suffered among kokanee salmon, coho salmon, and rainbow trout. In 1998, re-emerging OMVD was found in rainbow trout cultured in central Japan. OMVD has become a major problem in pond culture of rainbow trout in these areas. We were able avoid an outbreak of OMVD in masu salmon, kokanee salmon, coho salmon, and rainbow trout in Hokkaido and Tohoku district. OMV can be horizontally infected to coho salmon and rainbow trout through contaminated rearing water. OMV was sensitive to ultraviolet irradiation and iodophore treatment, and was inactivated in fertilized eggs. Although detection of OMV in carrier fish was difficult using PCR, this virus replicated and appeared in nervous tissue, kidney, liver, and ovarian fluid at the mature stage. All eggs and facilities were disinfected by iodophore just after fertilization and eggs were disinfected at the early-eyed stage. Eggs and fry were cultured in a virus-free environment. Formalin-killed vaccine against OMVD is effective for mature fish. The vaccine effectively blocks virus replication in ovarian fluid.

Diagnosis and Epidemiology of Goldfish Herpesvirus (CyHV-2) and Other Viruses with High Carrier Prevalence and Vague Clinical Signs

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Key words: CyHV-2, herpesvirus, aquareovirus, GCHDV, PCR

The herpesvirus of goldfish, (CyHV-2) and two aquareoviruses, *Aquareovirus C* and *Aquareovirus G*, also known as golden shiner virus (GSV)/grass carp hemorrhagic disease virus (GCHDV) and American grass carp reovirus (AGCRV) are widespread in cultured cyprinids. Diseases and mortality have been associated with these pathogens, but correlations between pathogen isolation and presumed disease outbreaks are weak. In the case of CyHV-2, it is clear that this is due to difficulty in culturing the virus and to the high prevalence of carrier states. With the aquareoviruses, there is the perception that they are easily cultured, but they are often found

in healthy-looking fish and only rarely cultured from fish dying with the clinical signs normally attributed to these pathogens (except for GCHDV of grass carp in China). In our work, we developed quantitative PCR methods for these pathogens and applied them to samples of healthy and diseased fish from commercial fish farms. In a CyHV-2 survey that included 35 farms and 30 cases of goldfish mortality not attributable to other pathogens, we found that the prevalence of healthy carriers with up to a few million copies per ug host DNA was very high, but that fish moribund with CyHV-2 disease often have from tens to hundreds of millions of copies per ug. Our study of the aquareoviruses is still underway, but correlations between GSV numbers and presumed disease in golden shiners are weak. By providing PCR diagnostic tools and estimates of the importance of these diseases, we enable farmers to determine if expensive control or eradication efforts are warranted.

Piscine Betanodavirus Induces Expression of Mx Protein Gene and Anti-viral Hemorrhagic Septicemia Virus Activity in Japanese Flounder Natural Embryo (HINAE) Cells

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Key words: VHSV, Mx protein, betanodavirus, ABV, IFN

We investigated the ability of an aquabirnavirus (= dsRNA) and a piscine betanodavirus (= ss+RNA) to induce Japanese flounder Mx (JFMx) protein gene expression (a putative marker of interferon production) and consequential anti-viral hemorrhagic septicemia virus (VHSV) activity *in vitro* using Japanese flounder natural embryo (HINAE) cells. Inoculation of HINAE cells with aquabirnavirus (ABV) led to the expression of the JFMx protein gene within a few hours after inoculation, followed by the occurrence of cytopathic effects (CPE). In contrast, inoculation of HINAE cells with piscine betanodavirus led to the expression of the JFMx protein gene but did not induce CPE. A subsequent challenge with VHSV, conducted to test whether the expression of the JFMx protein created an antiviral state in these cells, resulted in significant suppression of VHSV replication. However, when HINAE cells previously inoculated with betanodavirus neutralized by a polyclonal antibody were challenged, VHSV replication was not suppressed, suggesting that attachment of the nodavirus to HINAE cells essentially mediates the induction of JFMx protein gene expression.

Parabrachiella sp., a New Record of Parasitic Copepod in Cage-Cultured Mangrove Snapper (*Lutjanus argentimaculatus*) in the Philippines

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Key words: copepod, *Parabrachiella*, *Lutjanus argentimaculatus*, Philippines

Mangrove snapper (*Lutjanus argentimaculatus*) are cultured in the Philippines in earthen ponds and floating cages. With the intensification of the aquaculture industry, heavy losses due to diseases including parasites have become one of the current major concerns. Recently, cage-cultured mangrove snapper (mean TL = 12 cm; mean FL = 9.8 cm; mean BW = 25 g) from Igang, Guimaras, Philippines, displayed loss of appetite, lethargy, stunted growth, and gradual 5% daily mortality. The affected snapper had been cultured for six months in 5 x 5 x 3 m floating net cages, with a stocking density of 20 fish/m^3 and fed commercial pellets twice daily at 3-5% body weight. At the time of sample collection, water temperature was 28°C with salinity of 35 ppt. Affected fish collected for parasite examination (n = 31) harbored a lernaeopodid copepod on the gill rakers with a prevalence and mean intensity of 81% and 2, respectively. The copepod is identified as *Parabrachiella* sp., similar to *P. lata* recorded in *Acanthopagrus* spp. (Sparidae) from China, Australia, and Taiwan and an unidentified *Parabrachiella* sp. in *Sillago sihama* (Sillaginidae) from Malaysia and Hong Kong. This is the first record of *Parabrachiella* sp. from *L. argentimaculatus* in the Philippines and elsewhere. The parasite remained attached and alive even after exposure to freshwater bath treatment. A detailed morphological description of the present species is needed. *Parabrachiella* sp. has great potential to become a constant, thus an emerging, parasite.

Exposure to Multiple Stressors Increases Risk of WSSV Outbreak

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Key words: WSSV, stressors, *Penaeus monodon*

White spot syndrome virus (WSSV) has been a big problem to the worldwide shrimp industry for almost a decade. To investigate the factors that lead to WSSV outbreak, eleven ponds were stocked with WSSV-free shrimp, all within a one month period. Physicochemical parameters were monitored 2-4 times daily. Nine ponds were successfully harvested after 128-173 days of culture in spite of a WSSV infection in eight of them between days 103 and 127. A WSSV disease outbreak was experienced in three ponds 3-6 days after the virus was detected. The virus was detected in shrimp that were exposed to stress factors 200 or more times. Such factors include dissolved oxygen below 4 ppm, temperature outside the range of 28-32°C, pH greater than 8.3 in the morning or 8.5 in the afternoon, water transparency outside the range of 0.2-0.6 m, and water level less than 1 m. Further exposure to more stress factors in WSSV-infected ponds can cause an outbreak, with greater risk in younger shrimp.

Prevalence of Important Viral Diseases of the Wild Black Tiger Shrimp, *Penaeus monodon*, in the Philippines

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Key words: prevalence, *Penaeus monodon*, WSSV, TSV, MBV, IHHNV, YHV, GAV, Philippines

Due to the absence of a national shrimp domestication program, *Penaeus monodon* broodstock/spawners used in hatchery operations in the Philippines currently come from wild populations. Shrimp samples were collected from seven sites that serve as primary sources of broodstock/spawners. The samples were analyzed using polymerase chain reaction (PCR) to determine the prevalence of white spot syndrome virus (WSSV), taura syndrome virus (TSV), monodon-type baculovirus (MBV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), yellow head virus (YHV), and gill-associated virus (GAV) during the wet and dry seasons. WSSV was more prevalent in the dry season (10%) than in the wet (0.3%) but there was no difference between male (6% and 0%) and female (12% and 0.4%) in either the dry or wet season, respectively. A relatively high prevalence was observed in shrimps collected in shallow waters which might be attributed to the contaminated effluents that come from shrimp aquaculture activities and settle first in shallow marine waters. On the other hand, MBV prevalence showed no sea-

sonal (20% for dry, 9% for wet) or sex variation: prevalence was 18% for males and 20% for females during the dry season and 6% and 9%, respectively, during the wet. TSV was not detected in any site in both dry and wet seasons. IHHNV prevalence was 18% during the dry season and 14% during the wet season, i.e., 20% of the males in the dry season and 10% in the wet; 17% in females during the dry season and 15% in the wet. YHV was detected in 1% of the males during the dry season and only 0.8% of the females; it was not detected in any site during the wet season. GAV had a total prevalence of 23% during the dry season (16% for males, 26% for females) and 27% during the wet season (16% for males, 29% for females). Our study showed that WSSV, MBV, GAV, and IHHNV were already established in the local marine environment and that wild population of *P. monodon* in the Philippines are still TSV-free. YHV contamination was minimal and occurred in only two sites. Broodstocks/spawners from contaminated sites may serve as the main source of viral contamination and as carriers for the vertical transmission of the virus when they spawn. Our results may serve as guidelines to hatchery operators on where and when to source broodstocks/spawners until a national shrimp domestication program for developing SPF shrimp broodstocks is fully established.

Immunostimulation and Vaccination for WSSV Management in Shrimp, *Penaeus monodon*

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Key words: immunostimulant, vaccine, *Penaeus monodon*

Of all shrimp viruses with no known cure, WSSV has been the most detrimental to shrimp culture. An immunological approach is being used to develop a strategy for preventing WSSV in shrimp. Shrimps do not have an adaptive response equal to vertebrates and rely primarily on innate non-specific responses. But recent data show that they can be protected by a *quasi*-immune response that involves memory acquisition. In the present study, both aspects of the shrimp immune system were harnessed to develop a strategy that would have an impact on WSSV infection. Immunostimulants, including oligonucleotides with CpG motifs, DNA (DNAzol) extracts, and Bacillus Calmette Guerin (BCG), were administered by intramuscular injection and nutritional factors such as alfalfa, methyl methanesulfonate (MSM), and wheat glucan (WG) were given as feed additives, either singly or in combination with formalin-killed WSSV vaccine. Results showed that some immunostimulants given singly were able to increase the *in vitro* immune indices in shrimp. Cumulative mortality was significantly lower in shrimps given certain immunostimulants than in the control. Vaccination alone was able to increase shrimp survival up to 45 days post-infection with the live virus. There was a slight synergistic effect in survival when nutritional factors combined with formalin-killed vaccine were given orally and the shrimp were challenged via immersion. The use of immunostimulants and vaccines could be a viable approach to WSSV management in shrimp.

Regional Mechanisms for Recognizing Emerging Aquatic Animal Diseases in Asia-Pacific

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Key words: NACA, OIE Aquatic Animal Health Code, koi herpesvirus, grouper iridoviral disease, white tail disease, infectious myonecrosis, abalone viral mortality, epitheliocystis, akoya oyster disease, *Marteilioides chungmuensis*

The quarterly aquatic animal disease (QAAD) reporting system has been a useful mechanism for sharing aquatic animal disease information amongst 21 participating governments in the Asia-Pacific region. The NACA Asia Regional Advisory Group (AG) has often recognized the value of the regional reporting system and strongly recommends its continuation. The reporting system was developed following the recommendations of the NACA/OIE Expert Consultation in 1996 and has been a joint activity between NACA, FAO, and the OIE Regional Representation (Tokyo) since the second quarter of 1998. It was eventually integrated into the regional aquatic animal health program of NACA. The regional reporting system provides up-to-date information on important diseases in the Asia-Pacific region, serves as an early warning system for emerging diseases, and can be a valuable source of information to support risk analysis. To date, 36 reports have been published and disseminated.

Since 2001, the NACA/FAO/OIE QAAD list has been revised annually by the AG on Aquatic Animal Health to reflect changes made in the OIE list of aquatic animal diseases as shown in the latest edition of the OIE *Aquatic Animal Health Code*, and to include diseases of concern to the Asia-Pacific region. Important examples include:

"Mass mortalities of koi carp" in Indonesia in 2002 was listed under "unknown diseases of serious nature" in the FAO/NACA/OIE regional QAAD reporting list in the first quarter of 2003. From 2004 onwards, it was listed as "infection with koi herpesvirus (KHV)". In May 2006, the OIE International Committee adopted the listing of this disease for the 2006 edition of the OIE *Aquatic Animal Health Code.*

Recognizing the importance of iridoviral disease in terms of its potential to spread and cause economic loss, "grouper iridoviral disease" was listed in the QAAD under "any other diseases of importance," effective the first quarter of 2003.

Epitheliocystis, akoya oyster disease, and the molluscan pathogen *Marteilioides chungmuensis* were included in the QAAD list, effective the first quarter of 2003, to assist in collecting occurrence data.

Recognizing its potential to spread, "abalone viral mortality" was included as an unknown disease of a serious nature in the QAAD list, effective the first quarter of 2004. In May 2006, the OIE International Committee adopted the listing of this disease for the 2006 edition of the OIE *Aquatic Animal Health Code.*

White tail disease caused by *Mr*NV and XSV in *Macrobrachium rosenbergii* was included for reporting from the first quarter of 2005.

Considering the large scale introduction of *Penaeus vannamei* in the region, infectious myonecrosis was added to the QAAD list, effective for reporting from the first quarter of 2006.

At the General Session of the OIE in May 2007, the OIE Aquatic Animal Health Standards Commission proposed listing white tail disease and infectious myonecrosis in the 2007 edition of the *Aquatic Animal Health Code.*

At the fifth meeting of the AG on Aquatic Animal Health (AGM-5) held at the NACA Secretariat in Bangkok on 22-24 November 2006, the list was revised again. While doing so, it was agreed that all OIE listed diseases should be included in the regional QAAD reporting system. However, delisting of diseases by the OIE should not lead to their automatic delisting from the regional QAAD list because a globally delisted disease may still have relevance to the region. As a result of these revisions, the regional reporting system and the functioning of the AG have contributed significantly to recognizing emerging aquatic animal diseases in the region.

Surveys of Giant Freshwater Prawn Viral Diseases, *Mr*NV and XSV, in Thailand

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Key words: *Mr*NV, XSV, giant freshwater prawn larvae, risk factors

Giant freshwater prawn larvae (152 specimens) from hatcheries in Nakornpathom and Suphanburi provinces were collected and diagnosed for *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus-like particles (XSV) using RT-PCR during June 2006-February 2007. Of the specimens, 3.3-38.71% were infected, with a significantly lower number in February 2007. Infected larvae usually died 10 days after hatching. The affected larvae had lost appetite, were listless, and exhibited pale orange or dark coloration with mortality over 80%. The pathological changes related to the virus infection were basophilic cytoplasmic inclusions in the hepatopancreas and partial necrosis in the abdominal striated muscle. Electron micrograph of the viral pellets extracted from tissue homogenates of affected larvae revealed two sites of viral particles, 26-30 nm and 17 nm in diameter, which might respectively relate to *Mr*NV and XSV. Cloned and sequenced PCR products had 97-99% nucleotide and 98-100% amino acid homology to published sequences of *Mr*NV and 96-98% nucleotide homology and 96-99% amino acid homology to published sequences of XSV. Risk factors for *Mr*NV and XSV infection were analyzed; 'period of nursing' and '*Mr*NV/XSV presented in brooders' were significant factors associated with the spread of *Mr*NV/XSV in giant prawn larvae. Therefore, prawn brooders shall be screened before introduction into hatcheries. Nursing periods may relate to viral spread but further investigation is needed to understand the relationships between the spread of *Mr*NV/XSV in both brooders and their off-spring and seasonal variations.

Widespread Distribution of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) Infection in the Philippines: Implications for Farmed *Penaeus vannamei*

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Key words: IHHNV, penaeid shrimp, crustaceans, Philippines

The impact of infectious hypodermal and hematopoietic necrosis virus (IHHNV) infection on *Penaeus monodon* has generally been regarded as low. With the entry of *P. vannamei* in the shrimp farming scenario in the Philippines, however, there is a need for heightened awareness and vigilance towards the disease as it may cause runt deformity syndrome (RDS), mortality, growth retardation, and low fecundity among broodstocks. Screening of crustaceans for important viruses has increased since 2005 to know their prevalence and to identify emerging problems in the burgeoning culture areas of the introduced species. Viruses were identified using polymerase chain reaction (PCR) tests and histopathology. Results for 2005 and 2006 indicate IHHNV prevalence of 11% and 38%, respectively, in *P. monodon*, and 25% and 37.5% in *P. vannamei* for the same years. Wild shrimp and crab also carry the virus. Prevalence in hatchery-reared *P. vannamei* postlarvae for 2007 is more than 25%. The possible occurrence of RDS is being monitored in the growout using slaughter data. Although positive IHHNV results have been obtained in postlarvae, no RDS has been detected so far. Results of this study show an urgent need for proper management of *P. vannamei* so that IHHNV will not proliferate and create a new problem in its production.

Multi-Gene Sequence Analysis Distinguishes Ten Philippine *Vibrio* Isolates Pathogenic to Shrimp from Type Strains *V. harveyi* and *V. campbellii*

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Key words: *Vibrio harveyi*, *V. campbel*lii; toxR, hemolysin, lux gene, ornithine decarboxylase gene

Type strains *Vibrio harveyi* (NBRC15634) and *V. campbellii* (NBRC15631) exhibited 74% and 79% *toxR* and *hemolysin* gene sequence similarity, respectively. The complete 2,181 bp ornithine decarboxylase (*odc*) gene was isolated and sequenced from *V. harveyi* but no gene homologue was amplified from type strain *V. campbellii* using primers based on the *V. harveyi* sequence. Primers based on the complete 2,060-bp *V. harveyi luxAB* gene amplified a partial *luxB* gene fragment from *V. campbellii* but did not amplify a *luxA* gene homologue. Multilocus gene sequence analysis identified gene markers that could differentiate type strain *V. harveyi*

from type strain *V. campbellii*. Further, multilocus gene sequence analysis also revealed that ornithine decarboxylase and luciferase gene homologues were amplified and sequenced from all ten Philippine *Vibrio* isolates pathogenic to shrimp, with 96-98% sequence similarity of the *odc* and *luxAB* genes from type strain *V. harveyi*. Surprisingly, however, all ten isolates exhibited higher *toxR* and *hemolysin* gene sequence similarity with *V. campbellii* (at 92-93% and 92-97%, respectively) compared to just with 74-75% *toxR* and 82-85% *hemolysin* gene sequence similarity with *V. harveyi*. Using the *ornithine decarboxylase* and *luciferase* gene sequences as sole criteria, the ten Philippine *Vibrio* isolates could be classified as *V. harveyi*. If identification makes use of the *toxR* and *hemolysin* gene sequences, all ten isolates belong to *V. campbellii*. The multilocus gene sequence approach suggests that the pathogenic *Vibrio* isolates from the Philippines could be variant strains of *V. harveyi* that possess *V. campbellii*-like *toxR* and *hemolysin* genes.

PLENARY LECTURE

Epidemiology and Surveillance of Crustacean Diseases

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Key words: epidemiology, surveillance, crustacean diseases, Asia, shrimp, OIE

The history of crustacean aquaculture, and particularly shrimp farming, has been characterized by a great deal of health problems which have often lead to drastic declines in production and major economic losses. Researchers, governments, and the private sector often responded promptly to emergencies by developing diagnostic methods and health management strategies. Over the past decade epidemiological methods have played an increasingly important role in these efforts. Epidemiology is the study of diseases in populations. As such, epidemiological methods can be used to shed light on the frequency, distribution, and determinants (causes) of diseases as well as provide valuable information on the accuracy of diagnostic methods. An important epidemiological tool used for the systematic collection, analysis, and dissemination of health information is surveillance. In an attempt to increase the understanding of crustacean diseases and identify effective health management strategies for limiting losses, a great deal of effort has been spent over the years towards the development of passive and active surveillance systems for crustacean health problems. The World Organization for Animal Health (OIE) endeavors to provide improved guidance to member countries on the establishment of surveillance systems for aquatic animal diseases. In this presentation examples of the application of epidemiological methods and surveillance for the investigation and control of crustacean diseases will be provided. Potential strategies to strengthen capacity in epidemiology and surveillance of crustacean diseases in Asia will also be presented.

POSTERS

Immunomodulatory Effects of Supplemental Onion and Ginger on Brown Marbled Grouper (*Epinephelus fuscoguttatus*) Innate Immunity

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Key words: immunomodulation, brown marbled grouper, innate immunity

A 12-week feeding trial was conducted to evaluate the effects of different dietary immunostimulants to grouper, *Epinephelus fuscoguttatus*. Four experimental diets containing either onion, ginger, ß-glucan, or vitamin C and a control diet (without immunostimulants) were fed to duplicate groups of fish (40 fish/tank) with an average weight of about 44 g. Each diet was fed at 5% of the fish body weight per day, later adjusted to 3% given every 2 days. Weight gain increased significantly in fish fed the onion or ß-glucan diets compared to the control. Hematocrit was higher but not significantly different in the onion and vitamin C groups compared to the other treatments. Fish fed the diet supplemented with ginger produced the significantly highest total Ig. Production of superoxide anions (O^{-2}) and lysozyme activity tended to be higher but not significantly in fish fed the ginger or vitamin C diets. Among the leukocytes, lymphocytes (percentage) increased visibly in fish fed with onion or ginger. This study demonstrated that onion and ginger have immune modulating effects that could warrant their use as components of health-promoting grouper diets.

Parasitic Diseases of the Abalone (*Haliotis asinina*) in the Philippines

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Key words: parasites, *Haliotis asinina*, Philippines

Abalones, marine mollusks belonging to the genus *Haliotis*, are single-shelled herbivorous gastropods. Abalone culture is one of the fastest growing mollusk aquacultures and important economic activities in Southeast Asia. Donkey's ear abalone (*Haliotis asinina*) is the primary cultured species for the commercial abalone fishery in the Philippines. But, as mollusk aquaculture develops, one of the major concerns is the potential spread of parasites. This study aims to screen abalone (*H. asinina*) for the presence of parasites and to determine the host-parasite relationship through histopathology. Histopathological study of cultured abalone grow-out yielded first

time records of hemocyte and coccidian-like parasites: metazoans and ciliates. In hatchery-reared juvenile abalone, *Pseudoklossia*-like parasites were detected. Identification of these parasites is being confirmed.

Biodegradation of Monochloroacetic Acid (MCA) by a Presumptive *Pseudomonas sp*. Bacterium Isolated from Malaysian Paddy Field

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Key words: monochloroacetate, monochloroacetic acid, MCA, dehalogenase, biodegradation

Most halogenated compounds are major environmental pollutants. Studies demonstrate that monochloroacetic acid (MCA) is toxic to aquatic life such as fishes and, in particular, algae. Many soil microorganisms are capable of utilizing halogenated-substituted organic acids as their sole carbon source for growth. Organically bound halogen is liberated as halide ion. The most common use of MCA is in the production of phenoxy herbicide. Generally, MCA is converted to sodium chloroacetate and then reacted with 2,4-dichlorophenol to produce 2,4-dichlorophenoxyacetic acid (2,4D). Typically, concentrations of MCA in aquatic systems do not exceed 1 g/liter, but due to extensive use of certain herbicides in agricultural areas, its concentration may exceed this amount. Moreover, chloroacetic acid has a relatively longer half-life in the natural environment due to resistance to photodegradation. We isolated an MCA-degrading bacteria, tentatively identified as *Pseudomonas sp*., from a paddy field that can degrade MCA in concentrations ranging 5-40 mM. Quantitative agreement between the amount of MCA introduced and chloride released was also found. The results of our current study demonstrate potential use of *Pseudomonas* sp. as a suitable biological agent for biodegradation of MCA in contaminated agricultural areas.

Cloning, Characterization and Expression Analysis of a cDNA Encoding Granulin Gene of Nile tilapia (*Oreochromis niloticus*)

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Key words: granulin, cDNA, Nile tilapia, immune system

Granulins are a group of highly conserved growth factors that have been described from a variety of organisms spanning the metazoan. They have roles in multiple processes involved in cell growth, development, and wound repair in rodents and humans. In this present study, we isolated the full-length cDNA encoding Nile tilapia granulin gene by *in silico* cloning and 5'Rappid Amplification Complementary DNA Ends (RACE). The specific primer was designed from the partial sequence of granulin cDNA isolated from a cDNA library of Nile tilapia spleen. The complete cDNA sequence consisted of 1,196 bp containing an open reading frame (ORF) of 468 bp encoded 156 amino acid residues, including 16 amino acid residues of a signal peptide. Phylogenetic analysis clearly showed that Nile tilapia granulin was clustered in the same sister group of fish granulin genes. Expression analysis using RT-PCR revealed that high levels of granulin mRNAs were present in the spleen, intestine, gills, and kidney. Lower levels were found in the liver, muscle, heart, brain, skin, and stomach. Our data provide basic molecular information useful for further investigation of the function of the granulin gene in the Nile tilapia immune system.

Life Cycle of the Marine Leech (*Zeylanicobdella arugamensis*) Isolated from the Body of Sea Bass (*Lates calcarifer*) Under Laboratory Conditions

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Key words: marine leech, *Zeylanicobdella arugamensis,* sea bass, larvae, embryonic, development

Infestation of an unidentified marine leech in Malaysia was first reported in 1988 in grouper cultured in floating cages. During 2004-2006, the marine leech, *Zeylanicobdella arugamensis*, was regularly isolated from marine fish cultured at cages. In May 2006, approximately 60% of moribund sea bass fingerlings reared in cages were infected by *Z. arugamensis* which also served as a vector of the bacteria, *Vibrio alginolyticus.* There is little knowledge about the biology of *Z. arugamensis*, particularly from Southeast Asia. The aim of the present study was to determine

its life cycle under laboratory conditions. A total of 102 adult leeches, ranging 4.51-14.02 mm, from five trial experiments were sampled and bought into the laboratory. Leeches of ≥10.00 mm deposited cocoons after 5-8 h isolation and continued to deposit them until day 3. About seven days were needed for the new cocoons to develop into juveniles under 27°C at 28 ppt and another 9-10 days to reach the adult stage. Adult leeches deposited cocoons 24-72 h after they matured. A total of 31 juvenile leeches were introduced into 31 uninfected sea bass fry individually. Only 26 (83.87%) of the juvenile leeches reached the adult stage. In the present study, *Z. arugamensis* required 17-18 days to complete their life cycle.

Outbreak of Vibriosis in Mantis Shrimp (*Squilla* sp.)

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Key words: mantis shrimp, bacteria diversity, antibiogram

A disease outbreak in mantis shrimp (*Squilla* sp.) was reported to the marine hatchery of Universiti Malaysia Terengganu, Malaysia, in August and September 2007. Approximately 50 wild-caught mantis shrimps were bought from local fisherman and maintained in the hatchery. After a week, all the shrimps had black and brown circular lesions on the carapace and abdomen, melanization of the telson and uropod, and their eyes blackened. Twenty-four bacteria isolates from lesions on the carapace and abdomen were identified using a commercial identification kit (BBL Crystal, USA). The successfully isolated bacteria were *Vibrio alginolyticus*, *V. hollisae*, *V. mimicus*, *V. parahaemolyticus Weeksella virosa, Shigella* spp., and *Flavobacterium* spp. Antibiograms of the 24 bacterial isolates were also determined in the present study.

Surveillance of Bacteria Species in Diseased Freshwater Ornamental Fish from Aquarium Shops

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Key words: diseased ornamental fish, bacteria, antibiogram

Bacteria-infected freshwater ornamental fish from retail pet shops in Kuala Terengganu, Terengggganu, Malaysia, were surveyed from July to September, 2007. Diseased fish included dwarf gourami (*Colisa lalia*), discus (*Symphysodon aequifasciatus*), discus cichlids (*Symphysodon* spp.), black tetra (*Gymnocorymbus ternetzi*), swordtail (*Xiphophorus helleri*), platy (*Xiphophorus maculates*), variegated platy (*Xiphophorus variatus*), black ruby barb (*Barbus nigrofasciatus*), tiger barb (*Barbus pentazona hexazona*), Sumatra barb (*Barbus tetrazona*),

fighting fish (*Betta splendens*), guppy (*Poecilia reticulata*), mollies (*Poecillia* spp.), and silver catfish (*Pangasuis sutchi*). The bacteria were isolated using blood plates, cytophaga agar, GSP plates, XLD plates, and MacConkey without crystal violet. The isolated bacteria were identified using a commercial identification kit. Antibiograms of the isolated bacteria of the present study were also determined.

Herbal Extract Effects on White Spot Syndrome Virus (WSSV) in Shrimp (*Penaeus monodon*)

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Key words: herbal extract, WSSV, virucidal activity, *Penaeus monodon*, *Phyllanthus amarus*

Synthetic drugs and chemicals used in aquaculture cause disadvantageous side effects, while medicines made from medicinal herbs are non-toxic, easy to use, and pollution-free. Many medicinal herbs have potent antiviral properties. The extract of *Phyllanthus amarus* is a lignan composed of the compounds: niranthin, phyllanthin, and hypophyllanthin which have an impact on the white spot syndrome virus (WSSV) in the shrimp, *Penaeus monodon*. The virucidal activities of the three substances were tested by mixing them with WSSV, followed by injection into healthy shrimp. The quantity of WSSV DNA on the gills of tested shrimp was measured before and seven days after injecting the mixture. The quantity decreased significantly after injection. Anti-virucidal activities were also assessed by observation of the mortality rates of injected shrimp. The lignan compound inactivated the virus when injected in *P. monodon* at a dose of 100 mg per kilogram body weight. The survival rate of the lignan injected shrimp was 96.67%, compared to the positive control in which it was only 3.33%.

Phyllanthus amarus

WSSV infected on *Penaeus monodon*

Viral Nervous Necrosis Virus (VNN) Infection in Grouper Larvae in Southern Vietnam

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Key words: viral nervous necrosis virus, grouper, southern Vietnam

Groupers showing loss of appetite, floating near the surface, and corkscrew swimming were used to study the viral nervous necrosis (VNN) disease. Histopathological analysis revealed hemorrhage in the brain tissue and vacuolation in the retina. The liver had edema and many dead cells. Cytopathic effects (CPE) specific to VNN were observed in E-11 cells, 3-5 days after inoculation. Using the primer set for amplification of the RNA gene, RT-PCR produced 280 base pairs. Challenge was performed using intramuscular injection of the virus at $10^{8.5}$ $TCID_{50}$/0.1 ml/fish, which caused 78.3% mortality within four days after injection. VNN virus might be an important pathogen agent in grouper larvae in Vietnam.

Current Status of Fish Diseases in Cambodia

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Key words: fish diseases, Cambodia, carp, virus, bacteria, parasites

Aquaculture in Cambodia is gradually developing. Most farmers prefer to culture native fish species such as *Pangasius* spp., *Clarias* spp., *Channa* spp., and key freshwater cyprinids that are popular food fishes, have high market value, and adapt well to the local environment. However, national and provincial fisheries research and fish seed production stations/centers plus several NGOs are promoting extensive/semi-intensive culture systems consisting of low input ponds and rice-cum-fish or other integrated fish/animal/vegetable polyculture techniques to raise Chinese carp (silver, bighead, and grass carp), common carp, Indian carps (catla, rohu, and mrigal), and tilapia. Escape of these exotic fish species may impact natural aquatic resources and biodiversity in Cambodia. They could affect indigenous species through breeding, competition for feed and habitat, and disease, resulting in negative impacts on household income and the national economy. Based on the national list of priority diseases highlighted in the National Strategy for Aquatic Animal Health Management in Cambodia, the endemic and exotic pathogens that may infect cultured fishes include nine viral diseases, five bacterial diseases, and seven parasitic diseases. Updated information on fish diseases in Cambodia was published in the FAO/NACA Quarterly Aquatic Animal Disease Report (Asia and Pacific Region).

Monitoring Antimicrobial Usage in Marine Shrimp Farms

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Key words: drug resistance, *Vibrio*, marine shrimp farm, minimal inhibitory concentration (MIC), antibiotics, Thailand

Bacteria that are resistant to common antimicrobials used on shrimp farms in Thailand were monitored. A total of 395 *Vibrio* strains were isolated from moribund shrimp and rearing water in Samutsakhon, Samutsongkhram, Chachengsoa, Chontaburi, Rayong, Chantaburi, Trat, Songkla, Nakornsritammarat, Chumporn, Prajiuabkirikhan, Satun, Pang-nga, Phuket, and Trang Provinces during 2001-2004. Minimal inhibitory concentration (MIC) using agar method helped determine bacterial susceptibility to chloramphenicol (CP), erythromycin (E), furazolidone (FD), oxolinic acid (OA), oxytetracycline (OTC), norfloxacin (NFX), prefloxacin (PFX), trimethoprim (TM), sulfadiazine (SD), and sulfadimethoxin. The MIC breakpoints (ug/ml) of drug resistance were based on NCCLS interpretative guidelines. Results show that, during 2001-2003, a high number of *Vibrio* strains in all areas were resistant to SD (100%), E (92-100%), TM (61-100%), NFX, PFX, and FD (4-96%). An average of 53.75% and 17.67% strains were resistant to OTC and OA, respectively. The average percent of OTC-resistant strains gradually declined from 2001 to 2003, and rapidly declined in 2004. In 2003, only 6% of the strains were OA resistant while no strains were OA resistant in 2004. The bacterial samples that carried R-plasmid transfer were higher than in previous reports. Most of the resistant strains detected in 2001-2003 belonged to multiple drug-resistant strains while only monomer and double type drug resistance were detected in 2004. Our results provide necessary information on the impact of drug usage which may be caused by misuse and non-compliance among users. More attention should be paid to research into the pharmacokinetics of antibiotics in shrimp culture and the monitoring of the drug resistance program. This study was partly supported by the Japanese Trust Fund, SEAFDEC.

Status of Aquatic Animal Diseases in the Philippines

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Key words: aquaculture, aquatic animal diseases, health management, disease surveillance

The occurrence of aquatic animal diseases is one of the major constraints in the growth of the aquaculture industry in the Philippines. Significant economic losses due to diseases were experienced among cultured species such as shrimp, tilapia, grouper, and others. The Bureau of Fisheries and Aquatic Resources (BFAR) programs on fish health and its continued efforts have made the industry aware of the importance of health management in the sustainability of the industry. BFAR established fish health laboratories in different regions to provide accessible

diagnostic services and technical assistance. The disease surveillance and reporting system has been an effective tool to the fish health network for disseminating information on disease occurrences in different regions in the country and serves as the basis for the formulation of policies and regulations regarding the in-country movement of aquatic animals. Risk analysis is conducted for imports that may present risks of entry of exotic diseases into the country. This paper will provide information on economic diseases of fish and shrimp, and aquatic animal health management strategies in the country.

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Key words: Lao PDR, fish diseases, aquaculture

Aquaculture development in Lao PDR has traditionally been based on lessons learned from neighboring countries such as China, Vietnam, and Thailand. Fish seed farms were built in many provincial capitals during the Indochina war period, especially during the 1960s with USAID assistance in Vientiane, Savannakhet, Pakse, Sayaboury, and Luang Prabang. In the early 1970s, hatcheries were constructed in northern provinces (Houaphanh, Xiengkhouang, and Oudomsay) with the assistance of China and Vietnam. From 1997 onwards, a number of external donors, particularly the FAO/UNDP, have been assisting the Lao government in aquaculture development such as capacity building, extension, fish seed production demonstration, fish culture techniques, information on technologies, rehabilitation of hatcheries, etc. By the end of 2001, Lao PDR had 30 hatcheries throughout 18 provinces, 17 of which belong to the provincial government and 13 of which belong to private farms, plus 9 new hatcheries under construction.

Indigenous species include *Barbodes gonionotus (Pa park), Channa miclopeltes (pa doe), Hampala macrolepidola (pa soud), Hemibagrus numerus (pa kod), H. wyckioides (pa kheung), Pangasius kremfi (pa souay), Wallago leeri (pa khoune), W. attu (pa khao), W. dimina (pa khop), Osteochilus melanopleurus (pa nock khao), Cirrhinus molitorella (pa keng), C. microlepis (pa phone), Labeo behri (pa va), Morulius chrysophekadion (pa phia), Probarbus jullieni (pa eun), Clarias batrachus (pa dukna)*, and *Osphrosnemus exodon (pa men)*. Exotic species include the Indian major carps (*Labeo rohita, C. mrigala, Catla catla,* and *Cyprinus carpio)* and some Chinese carps (*Hypothalmichthys nobilis, H. molitrix, Ctenopharyngodon idella*, and *Oreochromis nilotica).* In the Aquaculture of Indigenous Mekong Species Project (AIMS), Lao PDR chose to study seven of the 17 indigenous species, namely *B. gonionotus*, *C. microlepis* (by km 08 Pakse station), *C. molitorella* and *P. jullieni* (by Nalouang station), *C. batrachus, M. chrysophekadion*, and *O. exodon* (by Nam Huang station).

Fish diseases in Lao PDR include epizootic ulcerative syndrome (EUS) in snakeheads, *Edwardsiella tarda*, *Aeromonas hydrophila*, *Vibrio* sp, parasitic infections (*Lernea* sp., *Gyrodactylus* sp., *Oodinium* sp., *Argulus* sp., *Epistylis* sp., *Columnaris* sp., *Icthyophthirius* sp.), and red spot disease. The Department of Livestock and National Animal Health Center collaborated with the Living Aquatic Resource Research Center (LARReC) that is in charge of this aspect. Since 1999, both organizations have considered this issue a most important research activity.

Antibiotic Susceptibility of Gram-Negative *Edwardsiella tarda* Isolated from Farmed Red Tilapia (*Oreochromis* spp.) Fingerlings

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Key words: antibiotic susceptibility, *Edwardsiella tarda*, tilapia fingerling

The use of antibiotics in aquaculture has been in much dispute over years. Not all antibiotics can readily safeguard farmed fish against bacterial causative agents such as *Edwardsiella tarda*. The current study focused on the antibiotic susceptibility of *E. tarda* isolated from farmed tilapia fingerlings using the rapid disc-diffusion method. Of seven tested broad-spectrum antibiotics, all isolates were susceptible to ampicillin and chloramphenicol but resistant to tetracycline. Antibiograms of the isolates from different geographical areas were almost similar except for susceptibility against sulphamethoxazole. The present study found that *E. tarda* isolated from farmed tilapia fingerlings were resistant to multiple antibiotics.

Molecular Characterization of *Edwardsiella tarda* Isolated from Farmed Red Tilapia (*Oreochromis* spp.) Fingerlings by RAPD-PCR

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Key words: *Edwardsiella tarda*, genetic diversity, wild-type phage M13

Tilapia farming is taking place almost everywhere. One of the bacteria isolated from tilapia is *Edwardsiella tarda*, which can cause Edwardsiellosis. Genetic diversity of this fish pathogen was investigated in the present study by randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Six to fifteen DNA fingerprints ranging 350 to 3000 bp were produced by *E. tarda* isolates using wild-type phage M13 primer. Genetic diversity of the isolates was accessed and the majority of the isolates were clustered together despite strain variability.

Quarantine, Surveillance, and Monitoring of Koi Herpesvirus in Singapore

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Key words: koi herpesvirus (KHV), Singapore, quarantine, ornamental fish, notifiable disease

The koi industry in Singapore is sizable. Thus, the introduction of any significant disease such as koi herpesvirus (KHV) is of great concern to the ornamental fish industry. Singapore's freshwater ornamental fish are imported and re-exported by traders licensed under the Accredited Ornamental Fish Exporters Scheme (AOFES). Under this scheme, Singapore ensures that only clean and disease-free fish are exported (including free from KHV). When detected by surveillance, notifiable diseases are reported in the Quarterly Aquatic Animal Disease Report (Asia and Pacific Region) of the OIE and to the Network of Aquaculture Centers in Asia (NACA). KHV is a new emerging disease of koi and carps, with potential to cause significant morbidity and mortality. As KHV is a serious threat in many countries, ornamental fish traders dealing with koi in Singapore are fully aware and concerned about the risks involved when importing this fish. In Singapore, the AVA has instituted compulsory inspection, testing, and quarantine of all koi consignments imported from countries where KHV has been reported or known to have been detected. In such cases, an initial sample is taken for KHV testing and the fish are quarantined for a minimum of three weeks. If initial results show positive for KHV, another sample is taken for confirmation. Koi consignments that are KHV-positive are destroyed, and the premises and equipment are disinfected. For high value koi, taking samples would be cost prohibitive to the traders. In such cases, the consignment is cohabited with sentinel KHV-free koi for one week, after which the latter are tested for KHV. The high value consignment continues its quarantine for three weeks. If the sentinel koi test negative for KHV, the consignment is released, but if the sentinel koi test positive, the consignment is destroyed. Strict controls are imposed to ensure that koi exports from Singapore are also free from KHV. These include a compulsory quarantine period of a minimum three weeks prior to the issuance of a KHV-free certificate.